THE GENETICS OF GONOPODIAL POLYMORPHISM IN TWO SPECIES OF POECILIID FISH

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1. Introduction

Natural populations of several species of *Xiphophorus* are known to be highly polymorphic for a variety of colour patterns that affect different parts of the body and fins (Kallman and Atz, 1966). Most of the patterns with the exception of the tailspot series are known to be under the control of sex-linked loci. The sex-determining mechanism of *X. variatus* and *X. milleri* is of the XX ♀♀-XY ♂♂ type, but in *X. maculatus* females of the same natural population may be either WY, WX or XX and males XY or YY (Kallman 1965*a*, 1970). The gonosomes of the three species are homologous (Kallman and Atz, 1966). The sex-determining mechanisms of the remaining five species of *Xiphophorus* are not well understood. The black macromelanophore patterns are in general equally strongly expressed in males and females, but some of the red patterns are poorly developed in females or not at all (Kallman, 1970). Least known is a polymorphism of *X. maculatus*, *X. variatus variatus*, *X. v. xiphidium* and *X. milleri* that affects the coloration of the gonopodium, the male copulatory organ. Although the study of *Xiphophorus* began in the late 1920's the only references to such a pattern are found in Gordon (1931), Miller and Minckley (1963) and Rosen and Kallman (1969). Some males of all four forms have a gonopodium that appears conspicuously black from a distance and contrasts sharply with the dull olivaceous grey coloration of the body. Apparently this pattern has no counterpart in the unmodified anal fin of the female. The transformation of the anal fin into a gonopodium is under the control of androgenic hormone (Grobstein, 1948). The structure of the gonopodium and its use during courtship and mating has been discussed in detail by Clark, Aronson and Gordon (1954), Franck (1964), Gordon and Rosen (1951) and Rosen and Gordon (1953).

We should like to call this component of polymorphism (and also others of *Xiphophorus*, see Kallman, 1970) to the attention of ethologists in the hope that they may determine whether one or the other colour variant possesses a selective advantage during courtship or agonistic behaviour. The present report describes the inheritance of black gonopodium (Gn) in *X. milleri* and *X. v. variatus*.

2. Material and methods

The stock of *X. milleri* (CL) listed in table 1 was derived from fish collected in Lake Catemaco, Veracruz, Mexico, in 1963. Male 1543-11 (table 2) was received in December 1963 through the courtesy of Dr R. R. Miller,

University of Michigan. He obtained his stock of *X. milleri* from Dr R. L. Dressler, who collected the fish in Lake Catemaco earlier that year. Two preserved collections of *X. milleri* have been examined (University of Michigan Museum of Zoology #177310, 184556).

The variable platyfish, *Xiphophorus variatus variatus*, listed in table 7 were collected at a number of locations in the Rio Tamesi drainage (Rios Sabinas, Guayalejo and Boquilla) in east-central Mexico. Female 2111-2 was captured at the Nacimiento of Rio Sabinas (Arroyo La Flor) and 2109-1 was obtained from the Arroyo Sarco, a small tributary to the Arroyo Encino that flows into the Rio Sabinas about 6 km. from its source. Fish of pedigree 2113 and 2115 came from two similar arroyos 3 km. north of the village of Encino. Fish of pedigree 2116 were caught in the Rio Guayalejo at Jaumave. All of the above fish were collected in February 1967. The VT stock is of mixed origin. It has been derived from fish obtained in 1957 at an unknown location in the Rio Tamesi drainage; the descendants were subsequently hybridised with individuals collected in 1965 in the Rio Boquilla. Most of these locations have been described in some detail by Borowsky (1969) and Darnell (1962).

Pigment patterns besides Gn referred to in this paper are Sv (spotted-ventral) of *X. milleri* and P^1 and P^2 (punctatus) of *X. v. variatus*. The three patterns are caused by macromelanophores and are sex-linked (Kallman and Atz, 1966). For details concerning gonopodial structure the reader is referred to the papers by Gordon and Rosen (1951) and Rosen and Gordon (1953).

Maintenance of fish has been described previously (Kallman, 1965*a*), but since the growth rate of Gn and non-Gn fish in *X. milleri* has been examined (see tables 5 and 6 below), it is important to go into some detail. Matings are set up in 15-litre aquaria. If the size of the brood is in excess of 12 young, the fry are spread out into similar tanks, but never more than 10-12 fish together. At 6 weeks *X. milleri* in our laboratory begin to differentiate sexually and the sexes can be told apart by the differently shaped anal fins. In males it is smaller and more triangular, but the third, fourth and fifth anal fin rays (referred to as the 3-5 complex) have not yet begun to elongate. At this time males and females are separated and recombined but again never more than 12 fish are kept together. After sexual maturation has been attained males of successive broods may be combined in 28-litre tanks. This is the reason why some sibs may grow up together from the time (6 weeks) that sexual differentiation commences, although they were raised in different aquaria during some of the first 6 weeks. Males of *X. milleri* may be sexually mature (capable of siring offspring) at the age of $2\frac{1}{2}$ months. At the age of 6 weeks Sv and Gn fish cannot be told apart.

The standard lengths of the fish were measured with vernier calipers with the aid of a low-power dissecting microscope to insure consistent placement of the calipers. The expression of Gn is variable. Fishes in a group were ranked by successive visual comparison with respect to the degree of gonopodial pigmentation. When the degree of pigmentation of two fishes was too close for a distinction to be made, tied ranks were assigned.

3. Results

The CL stock of *X. milleri* has been bred in the Genetics Laboratory for 15 generations comprising 22 crosses of the type + ♀♀ × Sv ♂♂, all but two

were brother-to-sister matings. With few exceptions Sv is inherited by males only (table 1) suggesting Y-linkage (Kallman, 1965*b*; Kallman and Atz, 1966). The 10 Sv females represent either cross-overs between the X and Y chromosomes or exceptional females with the sex chromosome constitution XY. Such XY females are also known from *X. maculatus*, *Poecilia reticulata* and *Oryzias latipes* (for references, see Kallman, 1968). The genotypes of the two Sv females tested were XY (see below). None of the fish listed in table 1 developed a black gonopodium.

TABLE 1

Inheritance of Sv in the CL stock of Xiphophorus milleri

Parents	++ ♀♀	×	Sv ♂♂
Offspring*	348 ++ ♀♀	10 Sv ♀♀†	317 Sv ♂♂

* Summary of 15 generations involving 22 crosses.
† 1 Sv ♀ each, 4th, 12th and 13th generation; 7 Sv ♀♀ 6th generation.

The data represented in table 2 are consistent only with the assumption that Gn is Y-linked and shows incomplete penetrance. Most relevant are pedigrees 1784 and 1983 through 2357 which represent essentially backcrosses of Gn males to the CL stock without Gn. If Gn were an autosomal trait, only one-half of the males of each generation is expected to develop a black gonopodium. This is clearly not the case (120 Gn : 49 +). Only 4 of 19 males of pedigree 1602 were scored as wild-type. If male 1543-11 was homozygous for Gn (either sex-linked or autosomally inherited), about one-half of the male offspring of pedigree 1717 should have exhibited Gn.

TABLE 2

Inheritance of black gonopodium (Gn) in Xiphophorus milleri

Parents*		Pedigree of Offspring	Offspring			
			♀♀	♂♂		
♀♀	♂♂		+	Sv	Gn	+
CL^3	1543-11 Gn†	1602	26		15	4
1602-1	CL^3 Sv	1717	37	40		
CL^3	1602-11 Gn	1784‡	55		54	7
1784-1	1784-11 Gn	1831	17		31	1
1784-3	1784-13 Gn	1835	29		16	1
CL^5	1835-11 Gn	1902	18		16	1
CL^6	1902-11 Gn	1983	10		9	6
CL^6	1902-12 Gn	1998	3		6	1
CL^8	1983-11 +§	2083	19		18	9
CL^8	1983-12 Gn	2121	13		10	7
CL^8	1983-13 Gn	2125	7		5	5
CL^{10}	2125-11 Gn	2260	16		8	1
CL^{10}	2121-11 +‖	2289			1	1
CL^{11}	2260-11 Gn	2337	20		4	6
CL^{11}	2083-11 +¶	2357	15		5	6

* Superscript refers to inbred generations of CL stock (table 1).
† The original Gn male.
‡ The 2nd brood of 23 ♀♀ and 30 ♂♂ was not listed by Kallman and Atz (1966).
§ 12 months. ‖ 13 months. ¶ 15 months.

Instead, none showed the trait. The results, however, are expected if Gn is Y-linked. Moreover, three phenotypically wild-type males of the Gn line when mated to CL females gave rise to Gn offspring indicating that they carried Gn non-expressed.

Particularly pertinent are those crosses that involve rare Sv females. We assign to them the XY genotype, since when mated to Gn (XY) males, offspring was obtained in a ratio of 1 ♀♀ : 3 ♂♂ (ped. 1832, 2521 : 13 ♀♀, 27 ♂♂, $\chi^2 = 0{\cdot}68$, $P > 0{\cdot}5$; table 3). Moreover, with a single exception

TABLE 3

Inheritance of Sv and Gn in Xiphophorus milleri
(Crosses involving XY females)

Parents ♀♀*	Parents ♂♂	Pedigree of Offspring	Phenotype of Offspring ♀♀ Sv	♀♀ +	♂♂ Sv	♂♂ SvGn	♂♂ Gn	♂♂ +
CL[4] Sv XY	1784-12 Gn XY	1832	—	4	2	2	2	—
CL[5] + XX	1832-11 Sv Gn YY	1891	10	—	27	1	25	4
CL[12] Sv XY	2337-11 Gn XY	2521	1	8	10	5	4	2
CL[13] + XX	2521-11 Sv Gn YY	2602	—	—	1	—	—	1
CL[13] + XX	2521-12 Sv Gn YY	2660	—	—	4	—	3	2
CL[14] + XX	2521-13 Sv Gn YY	2669	2	—	17	—	18	5

* Superscript refers to inbred generation of CL stock.

females were wild-type (XX) while male offspring consisted of four phenotypic (three genotypic) classes: Sv Gn males have two Y chromosomes, Gn males and presumably the + class in which *Gn* remained unexpressed are XY_{Gn}, most Sv males are XY_{Sv} but some could have been YY in which Gn did not manifest itself.

Further evidence for Y-linkage of Gn and for the XY genotype of the Sv females is provided by crosses using Sv Gn males (YY). They sired 49 Sv male offspring (XY_{Sv}), 46 Gn and 12 + males (XY_{Gn}) and one Sv Gn male (peds. 1891, 2602, 2660, 2669, table 3). The segregation of Sv and Gn in all but one fish is excellent proof that both Sv and Gn are Y-linked. If the Sv Gn male is the product of a cross-over, it would be proof that Sv and Gn are not allelic. Unfortunately, this fish was not tested.

No female offspring are expected from YY males; however, there were 12 Sv females (10 per cent.). Presumably they have the XY genotype and arose by the same unknown mechanism that is responsible for the occurrence of Sv females in the CL stock. It may be significant that only Sv females were present in pedigrees 1891 and 2669 while all XY_{Gn} fish were males. This suggests that whatever factors are responsible for occurrence of XY females interact with the Y chromosome marked by Sv and not with the one marked by Gn.

Since Gn is Y-linked in our *X. milleri* stock, it cannot be determined whether a similar pattern can develop in the unmodified anal fin of females in the absence of androgenic hormone. Evidence from other species of *Xiphophorus* suggests that Gn has no counterpart in females. No black pigmentation has been detected in females of *X. variatus* known to have inherited Gn (see below). Similarly, no pattern equivalent to Gn has been

seen in females of preserved collections of *X. maculatus* in which some males exhibit this pattern and in which the Y chromosome is known to be inherited in females. At best the problem can be approached in *X. milleri* by castrating immature males. At the age of 9 months none of four castrates had developed the pattern whereas 10 of 15 controls exhibited Gn at the age of 4 months and this score increased to 13 of 15 at 6 months (table 4). Three of the castrates received a large amount of methyltestosterone at the age of 9 months. Within 30 days after hormone administration and within 2 weeks after gonopodial transformation was complete, two of the three fish developed Gn (table 4). Thus, in the absence of male hormone, or alternatively in the absence of the transformed anal fin, Gn is not expressed in *X. milleri*.

TABLE 4

Hormonal control of the pattern "black gonopodium" in Xiphophorus milleri

No. of fish	Treatment	Age	Results
10 ♂♂*	control	—	7 Gn at 4 months; 1 Gn at 5 months; 1 Gn at 6 months; 1+ at 18 months
5 ♂♂†	sham op.	5 weeks	3 Gn at 3 months; 1 Gn at 5 months; 1+at 9 months
4 ♂♂‡	castrated	5 weeks	4+ with unmodified anal fin at 9 months§
3 castrated ♂♂§	methyl test.	9 months	2 Gn 30 days after treatment§‖; 1 + 68 days after treatment¶

* The first brood of pedigree 2260 (listed in table 2) and one fish of second brood (not listed in table 2).

† One fish of second and four fish of third brood.

‡ Four fish of second brood.

§ These are the same fish; one died at beginning of hormone injection. Hormone administration terminated after eighth injection on 30th day.

‖ Gonopodial transformation complete in one fish after 14 days; five injections of 5 μl (55 μg m.t.) each.

¶ Gonopodial transformation complete in two fish after 20 days; six injections of 5 μl (25 μg m.t.) each.

The expression of Gn in *X. milleri* is highly variable. It may range from a gonopodium that is entirely black including the specialised distal segments of the 3-5 complex to one in which merely 6-7 large pigment cells were present in the middle of rays 3 or 4. This latter pigmentation can only be recognised under the microscope. Such fish have been scored as wild-type (*e.g.* three of four + males of ped. 1602), but presumably even these pigment cells are controlled by Gn, because no such cells have ever been seen, although searched for, in males of the CL stock or in Sv males of pedigree 1717. The area most consistently covered by Gn is the middle of the shaft (rays 3, 4 and 5) while the immediate proximal and distal portions of the gonopodium (in that order) are black only in the most heavily pigmented fish. In a few isolated cases a dense accumulation of pigment cells was present only at the very base of the fin rays, a pattern that is identical with Gn of *X. v. variatus* (see below). There is no preferential aggregation of pigment cells along the ventral edge of ray 3 as in *X. v. variatus*. In a number of *X. milleri* the darkest pigmentation is found on the "spoon", the trough-shaped area that is formed by the distal halves of rays 5*a* and *p*. Gn in *X. milleri* does not develop until after sexual maturity has been attained. Approximately 70 per cent of the males develop Gn within 2 months after gonopodial transformation is complete; in the remaining males the pattern

may not appear until they are 20 months old and in some 2-year-old fish Gn remained unexpressed.

The frequency of Gn in natural populations of *X. milleri* must be exceedingly low, since this pattern was not represented in the two collections examined (94 mature ♂♂, 11 immature ♂♂).

A correlation has been found in *X. milleri* between the expression of Gn in a male and its size relative to those of the other males in its group (aquarium). The larger a fish is the more likely it is to express Gn and the more likely its expression will be good. The groups of males (A-N, table 5) are ranked according to the degree of Gn pigmentation and the standard length of each fish is also given. Since growth rate in fish is notoriously variable and may be influenced by genetic factors, brood size, crowding, light intensity, etc., only those males are included in table 5 that meet the following criteria: (1) all males must be of the same brood; (2) all males were raised together either throughout their entire life-span or since the time when gonopodial transformation began (see methods); (3) all males were sacrificed at the same time or nearly so. This is one of the reasons why the number of males listed for each pedigree in table 5 is less than the numbers for the same pedigree in tables 2 and 3. A member of a group may also have died or was sacrificed or removed for other reasons before it was measured and scored for the degree of Gn pigmentation. Thirteen of 14 Spearman's Coefficients were positive (table 5). The two-sided probability of this being the case if the two factors were not correlated is only 0·0018 (calculated from a binomial distribution where $P = 0{\cdot}5$). The median correlation coefficient of the 14 groups is 0·397 and 95 per cent. confidence limits on the true value give a range of 0·241 to 0·8 (median test). Our interpretation is that there is a weak, but consistent and significant correlation between size and pigmentation of the gonopodium.

Gn males, regardless of whether or not they show the pattern, tend to be larger than Sv (non-Gn) males. A direct comparison of their sizes was possible in one pedigree (2669) in which Sv and Gn males were raised together. It should be recalled that when the fish were arranged into the five groups (see methods), Sv and Gn fish could not be told apart. Thus they are combined randomly. The sizes of the Sv males are listed in footnotes 3-7 of table 5. The difference in size between Gn (larger) and Sv (smaller) males is statistically significant at the 0·01 level (table 6). The test used, a χ^2 analysis of a four-fold table, is conservative and nonparametric.

In pedigree 2602 (table 3) only a single brood of two fish was obtained. The Gn male (pattern not expressed) was 25 mm., the Sv male 19·8 mm. Both fish had been raised together throughout the entire life until sacrificed at 9 months.

Gn and Sv males segregated also in pedigree 2660 (table 3). These fish, however, could not be included in table 6, because the two classes were neither sibs nor reared together, although raised under similar conditions. The first brood of this pedigree consisted of five fish, all Gn, the second brood of four fish, all Sv. Nevertheless, it is interesting to note that again Gn males tended to be larger (see I, table 5) than those with Sv (22·9, 22·5, 22·0, 18·0).

For similar reasons the sizes of Gn and Sv males of pedigree 1891 could

not be included in table 6. This pedigree was made up of four broods, but after sexual maturity had been reached, all males of the first two broods were combined as group A and the fish of the last two broods as group B. Thus, whatever difference in size attributable to their being reared initially in different aquaria is obscured. Nevertheless, the size difference between the two classes of males is quite striking (two-sided, P = 0·0037, Wilcoxon's T test performed on the combined data; $T_{27,\ 28} = 581{\cdot}5$) and again point to a large median size of Gn males. The median size of Sv was 18·9 mm. and of Gn 20·6 mm. Thus not a single pedigree or group is known in which the Sv class is larger in size than the Gn class.

The character black gonopodium of *X. v. variatus* is also inherited as a sex-linked trait. The first three crosses listed in table 7 represent fish without the genetic basis for black pigment on the gonopodium. In addition a stock of *X. v. variatus*, VT, also derived from the Rio Tamesi drainage, has been raised in the laboratory for 13 years, but no male ever developed Gn. The two Gn males (2113-11, -12) sired only offspring with clear gonopodia. These results suggests that Gn may be sex linked and located on the X chromosome. Accordingly, females of pedigrees 2123 and 2387 must carry on one of their X chromosomes Gn. Both pedigrees 2343 and 2250 show this assumption to be correct. Especially telling is the result of pedigree 2343, because the male parent came from a line from which Gn was known to be absent.

The results of pedigree 2126 are consistent only with the assumption of X-linkage for Gn. The difference in frequency of Gn between the sexes (all androgen-treated females were Gn while only one-half of the adult males exhibited this trait) rules out both autosomal and Y-linkage. It follows that some of the females of pedigree 2126 must be homozygous carriers for Gn, and pedigree 2342 sired by a male of a line known to be free of Gn illustrates such a case. All X chromosomes of fish of pedigree 2335 and their descendants are marked by Gn (table 8).

The expression of black gonopodium in *X. v. variatus* is also highly-variable. It may range from a condition in which the intense black pigmentation is restricted to the very base of rays 3-7 to one in which also the spoon of ray 5 and the ventral (forward) edge of ray 3 immediately proximal to the spines may be black. In the darkest gonopodia the entire forward edge of ray 3 may be covered with melanophores but the most intense pigmentation is always present adjacent to the spines. The two heavy concentrations of melanophores in the distal part of the gonopodium coincide with the areas where the integument is thickest; during erection both areas come together and form a single black mark facing forwards and upwards, when the distal part of the gonopodium forms a temporary tube.

Even in the gonopodia with the strongest Gn expression few pigment cells are found between the spoon and the ventral edge of ray 3 and along the middle of rays 3-5. The pattern develops first at the base of the gonopodium and this is also the part of the fin where the pigment cells appear in androgen treated females. The first sign of the pattern may be visible before gonopodial transformation is complete.

4. Discussion

The character black gonopodium is represented in four of the eight species of *Xiphophorus* (Rosen and Kallman, 1969). It is absent from species

TABLE 5

The relationship between relative size (standard length, mm.) and degree of gonopodial pigmentation within broods of X. milleri

Order of gonopodial pigmentation in a group (#1 = the most heavily pigmented fish	Group code letter[1]													
	A	B	C	D	E	F	G	H[2]	I	J[3]	K[4]	L[5]	M[6]	N[7]
1	21·2	**23·8**	**26·3**	**20·7**	19·4	23·3*	22·8	**21·4**	**25·5**	**22·7**	23·4	**25·5**	**22·0**	22·3
2	**22·0**	**21·2**	**25·9**	**21·9**	**22·2**	**25·2**	**24·3**	19·8	**23·9**	**23·0**	**24·8***	24·8	**23·3**	**23·7**
3	**22·7**	**23·0**	22·4	**23·0**	20·2	21·9	**24·2**	**22·0**	22·5*	22·2	24·0		20·6	**23·4**
4	**22·0**	21·0	23·8	20·0*	**19·7**	**24·1**	22·8	20·0	22·1	18·6	**25·6**		18·2	22·1
5	20·8	**23·1**	**25·6**	19·9	20·1	22·8	**22·9**	**20·1**	21·4					**23·7**
6	**22·2**	**22·0**	23·4	18·2			22·7	**21·8**						20·9
7	19·9	20·9	24·2	19·9				**21·0**						22·8*
8	20·1	19·7	**24·6**					**20·3***						
9	20·7	19·9	**25·6**					19·6						
10	**22·0**	21·1	22·5					19·5						
11								19·3						
Spearman's rank difference correlation coefficient. Computed for size *vs.* pigmentation rank.	0·394	0·694	0·361	0·795	0·125	0·125	0·400	0·375	1·000	0·800	−0·850	1·000	0·800	0·241

TABLE 5—*continued*

An analysis of the above data]

Was a fish over median in size?

Did a fish have more than the median amount of gonopodial pigmentation?	YES	NO	Totals
YES	29	12	41
NO	12	29	41
Totals	41	41	82

χ^2 with Yates correction for continuity = 12·48. d.f. = 1, $P < 0{\cdot}005$.

[1] Explanatory notes: Sizes larger than the median size in a group are in bold type, and in groups containing an odd number of fish, the median is denoted by *. Median fish are not considered in the 2×2 contingency table. The pedigrees (age, months) of the groups are: A-1784 (4·5); B-1784 (4); C and D-1831 (6); E-1902 (9); F-1902 (9·5); G-1998 (12); H-2357 (4·5); I-2660 (7); J, K and L-2669 (5); M and N-2669 (6). " { " denotes tied ranks with respect to pigmentation.

[2] The last six fish showed no trace of Gn pigmentation. 20·3 rather than 20·1 was considered as the median in order to retain equal numbers below and above median size.

[3-7] Other sibs not having the gene for Gn were raised in the groups (see table 3). The non-Gn fish were not considered in the 2×2 contingency table. There sizes were:

[3] 21·9, 21·8, 20·9, 20·4. [4] 21·7, 21·1. [5] 23·1, 22·9. [6] 21·8, 21·2, 21·1, 21·0, 21·1, 19·8, 17·2. [7] 21·0, 20·5, 20·4.

TABLE 6

A comparison of size of Sv and Gn males of X. milleri (*ped. 2669*)
(*Sv* versus *Gn males and size above* versus *below median* within *a tank**)

	Sv	Gn	
Larger than median size in home tank	4	15	
Smaller than median size in home tank	13	6	χ^2_1 with Yates' correction is equal to 6·8123 with one degree of freedom. P = 0·01.

Since the comparisons are made within the tanks, between tank size difference effects are nullified.

* Their sizes (standard length, mm.) have been listed in table 5.

TABLE 7

Inheritance of Gn in Xiphophorus variatus variatus

Parents and phenotype*					Patterns of offspring									
					P²		+		Gn		P² Gn		P¹ Gn	
♀♀		♂♂		Pedigree	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
2109-1	P²	unknown		2110	2	6	—	—	—	—	—	—	—	—
2110-1	P²	2110-12	P²	2185†	16	23	12	—	—	—	—	—	—	—
2185-1	+	2185-16	P²	2378	9	15	—	—	—	—	—	—	—	—
2111-2	P²	2113-11	Gn	2123	2	5	4	3	—	—	—	—	—	—
2151-5‡	+	2113-12	Gn	2387	—	—	18	12	—	—	—	—	—	—
2123-1	+	2185-13	P²	2343	—	4	11	—	—	—	—	11	—	—
2123-2	+	2123-11	P²	2250	9	—	—	2	—	4	—	—	—	—
2115-1	+	2115-11	Gn	2126	—	—	50	37	15§	41	—	—	—	—
2126-7	+	2185-15	P²	2342	—	—	16	—	—	—	—	13	—	—
2126-5	+	2126-11	Gn	2335	—	—	16	1‖	—	14	—	—	—	—
2335-2	+	2265-11¶	P¹	2740	—	—	12	—	—	—	—	—	—	17
2335-1	+	2335-11	Gn	2703	—	—	11	—	—	13	—	—	—	—

* Their most likely genotypes are given in table 8.

† 5 ♀♀, P² or +, received each a total of 2 mg. of testosterone propionate in peanut oil in sixteen 5-μl injections over a period of 53 days. No Gn developed. These females served as controls for pedigree 2126.

‡ From 2115 ♀×2116 ♂.

§ Fifteen ♀♀ were hormone treated, all developed black pigment on modified anal fin. Twelve ♀♀ (7 months of age) injected twice with 5 μl (10 mg./ml.) of methyl testosterone in olive oil. Gn developed on the 9th day after the first injection. Three ♀♀ (5 months of age) received a total of 1·00 mg. of testosterone propionate in peanut oil in eight 5-μl injections administered over a period of 50 days. Gn developed during the course of this treatment.

‖ Fish died when 17 months old. ¶ From VT stock.

TABLE 8

Probable genotypes of parents listed in table 7

2109-1	X_P2^2		
2110-1	X_P2X_+	2110-12	X_+Y_P2
2185-1	X_+X_+	2185-16	X_P2Y_P2
2111-2	X_P2X_+	2113-11	$X_{Gn}Y_+$
2151-5	X_+X_+	2113-12	$X_{Gn}Y_+$
2123-1	$X_{Gn}X_+$	2185-13	X_+Y_P2
2123-2	$X_{Gn}X_+$	2123-11	X_P2Y_+
2115-1	$X_{Gn}X_+$	2115-11	$X_{Gn}Y_+$
2126-7	$X_{Gn}X_{x_n}$	2185-13	X_+Y_P2
2126-5	$X_{Gn}X_{Gn}$	2126-11	$X_{Gn}Y_+$
2335-2	$X_{Gn}X_{Gn}$	2265-11	X_+Y_P1
2335-1	$X_{Gn}X_{Gn}$	2335-11	$X_{Gn}Y_+$

with moderate to long swords. The trait is quite variable and there is some doubt as to whether the pattern in the different species and subspecies are strictly homologous (*i.e.* can be traced to a common genetic basis in an ancestral form or to a common developmental mechanism). As far as we know from a cursory survey of preserved collections of *X. maculatus* and *X. variatus*, males with black gonopodia occur in all river systems inhabited by the two species, but the only evidence for the presence of Gn in *X. milleri* is provided by male 1543-11 (table 2). In contrast to the variation of the three species, all males of *Xiphophorus couchianus gordoni* exhibit a Gn-like pattern.

The Gn pattern of *X. variatus* and *X. milleri* is presumably made up of macromelanophores *sensu* Gordon (1926, 1927), because upon close inspection of poorly pigmented gonopodia in which the outline of individual cells can be discerned, the Gn pigment cells are found to be larger and darker (more pigment) than the perivascular melanophores along the fin rays and those of the " stippled " type (micromelanophores) in the integument. Originally the black pigmentation of the *X. milleri* gonopodium was thought to be composed of micromelanophores (fig. 4, Kallman and Atz, 1966), but this is incorrect. This interpretation was based upon the examination of heavily pigmented gonopodia in which the pigment cells were so tightly packed that their individual shapes and size could not be appreciated. It must be emphasised, however, that no rigid definition of " macromelanophores " and " micromelanophores " exists. The term macromelanophore was first used by Gordon (1926, 1927) to describe the large, intensely black pigment cells of the sex-linked pigment patterns of *X. maculatus*. In contrast the term micromelanophores was applied to the smaller integumentary pigment cells that made up the background coloration and certain autosomal pigment patterns. Many—perhaps all—of the macromelanophore factors of *X. maculatus* give rise to atypical pigmentation in certain interspecific crosses while those of the micromelanophore series rarely do so. This definition of macromelanophore, encompassing the criteria of size, sex-linkage and enhancement after hybridisation while useful for *X. maculatus*, breaks down in certain other species of *Xiphophorus* as already noted by Becker (1965). Gordon (1931) thought that the black phase of the gonopodium of *X. maculatus* was composed of melanophores consisting of the " modified stipple type ".

Without exception females of *X. milleri* examined by us (the fish raised in the laboratory and the Michigan collections) possessed a heavy concentration of melanophores on the lower two-thirds of anal fin rays 3, 4 and 5. A similar pattern is not present in other species of *Xiphophorus*. These cells are similar in size and intensity of coloration to the stipple cells present in wild-type gonopodia. The black pigmentation in the anal fin of females is presumably a species specific trait and has nothing to do with the polymorphic character. An illustration of a female of *X. milleri* showing this pattern clearly has been published by Kallman and Atz (1966, fig. 4, the female in the upper-left corner). This pigmentation is visible also on the anal fin of the allotype (fig. 8, Rosen, 1960). In the absence of genetic information one could erroneously conclude that this aggregation of pigment cells represent the expression of Gn in females. If this were the case, however, all females of *X. milleri* would carry this factor and males with Gn would be expected in the CL line and in the preserved collections.

The expression of black gonopodium is under the control of androgenic hormone as shown by the hormone experiment with females of *X. variatus*. Thus Gn has to be considered as a variable secondary sex character which in turn modifies the coloration of another secondary sex character, the gonopodium or male intromittant organ. At least in *X. variatus* the development of the pattern is not contingent upon the structure of a gonopodium. The androgen-treated females do not possess a gonopodium; merely the distal parts of rays 3-5 are somewhat modified. However, in the intact animal the presence of androgenic hormone cannot be separated from the development of a gonopodium. Of the more than three dozen melanophore patterns known in the genus, Gn is the only one that is under hormonal control: X-linkage of Gn in *X. variatus* and Y-linkage in *X. milleri* are presumably fortuitous; no claim is made that the Gn locus of *X. milleri* is restricted to the Y chromosome. Because of the incomplete penetrance of Gn in *X. milleri* and its presumed lack of expression in females, cross-overs of Gn from the Y to the X chromosomes are virtually impossible to detect.

A second component of polymorphism affecting the anal fin, Anal-Red, has been described for *X. maculatus*, but no equivalent pattern is known from any other species. The intensity of the coloration of anal red is stronger in males than in females (Valenti, unpublished).

Since several species are polymorphic for Gn, the trait has presumably some adaptive significance. Because of the easily perceived difference between pigmented and unpigmented gonopodia (compare Sv + ♂, Fig. 4, with + Gn ♂, Fig. 5, Kallman and Atz, 1966), this polymorphism may play a role during certain types of behaviour. Unfortunately, no observations have been made on any stock of *Xiphophorus* with Gn. Fishes of this genus like other poeciliids are known to exhibit " gonopodial swinging " (for references see Franck, 1964), a deliberate flexion and rotation of the gonopodium in conjunction with one of the pelvic fins and accompanied by a peculiar asymmetrical S-curving of the body. The significance of gonopodial swinging is not well understood and no signal function has been ascribed to it. It is performed by isolated males (Clark, Aronson and Gordon, 1954), but its frequency is markedly increased by the presence of females. According to Franck (1964), it is performed in *X. helleri* only when females are present. Yet as various authors have observed gonopodial swinging is neither directed towards the female nor a prerequisite for successful copulation.

The differences in the pigmentation of the gonopodia are least noticeable when they are at rest. To our eyes gonopodial swinging performed by a black gonopodium is a strikingly conspicuous behaviour pattern easily observed over a considerable distance. The same movement by an unpigmented gonopodium is much less pronounced. If this behaviour has a signal function, such a signal could be considerably stronger when reinforced by coloration.

There is a significant trend of decrease in penetrance of Gn correlated with both passage of time and number of back-crosses to the CL stock for which no explanation can be offered. This trend, however, is reversed during the last two back-cross generations. The penetrance of Gn in pedigree 2669 (from CL^{11}, table 3) is the same as in pedigree 1602 (from CL^{1}, table 2).

Of particular importance, we believe, is the weak yet consistent correla-

tion in *X. milleri* (but not in the *X. variatus* pedigrees examined) between size of the fish and the intensity of the pattern and the size difference between Gn and Sv males.

The existence of two size classes within certain pedigrees of *X. milleri* is not a unique phenomenon for the genus. It has long been known to workers in this field (although unfortunately little has been published) that in many forms two types of males (small early maturing ones—" low males " and large late-maturing ones—" high males ", in German: Früh- und Spätmännchen.) are found when pure genetic strains are analysed. Since undoubtedly many loci affect size and maturation rate one should not expect to find clear-cut differences in wild populations or heterozygous stocks. Low and high males have been documented in *X. hellerii* (Peters, 1964) and *X. montezumae cortezi* (Zander, 1965) and Rosen and Kallman (1969) have illustrated the two extreme male types of *X. pygmaeus nigrensis*. Anders and Anders (1963) have reported that in *X. maculatus* (derived from the Jamapa population) XY males (heterozygous for Y-linked Sr) matured earlier and were smaller than YY males (homozygous for Sr). Many similar examples for *X. maculatus* have been found in our laboratory (Kallman, unpublished). In some instances the difference between the two types of males is of similar magnitude as the one between Sv and Gn males of *X. milleri*, but in other pedigrees the two classes are separated by a gap of several millimetres (size) and months (sexual maturity) with no overlap. Without exception the two types of males in pedigrees of *X. maculatus* can always be traced to a sex-linked factor (either X- or Y-linked). Whether the size difference is a pleiotropic effect of a pigment gene which serves as a marker or due to a second locus linked to it is not known. A situation in which different pigment patterns are correlated with adult size and the time of sexual maturation which indirectly affects the behaviour, provides ample opportunity for being maintained as a balanced polymorphism.

5. Summary

1. Several species of fishes of the genus *Xiphophorus* (Poeciliidae) are polymorphic for a black pigment pattern that affects the gonopodium, the male copulatory organ.

2. In *X. milleri* and *X. v. variatus* the pattern is controlled by a sex-linked locus. The gene for black gonopodium, Gn is Y-linked in the one stock of *X. milleri* examined and X-linked in a small sample of *X. v. variatus* from the Rio Tamesi.

3. The expression of Gn is quite variable and shows less than 100 per cent. penetrance in *X. milleri*. Females of *X. v. variatus* that carry Gn exhibit a clear anal fin. The pattern can be induced in them by the administration of androgenic hormone.

4. Gn has to be considered a variable secondary sex character which in turn modifies the coloration of another male secondary sex character, the gonopodium.

5. A correlation has been found in *X. milleri* between the expression of Gn in a male and its size relative to those of other males in its group. The larger a fish is the more likely it is to express Gn and the more likely its expression will be good.

6. Gn males regardless of whether or not they show the pattern tend to be larger than Sv (non-Gn) males.

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